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EXPERIMENTS IN FILTRATION OF ANTIHOG-CHOLERA SERUM *

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In accordance with the suggestions of Dorset and Henley[1] on the production of clear and sterilized antihog-cholera serum, we have attempted to produce bacteria-free serum by passage through Berkefeld V and Chamberland F. filters. The results of experiments in this connection have led to other investigations which are presented in this article.

The Chamberland F. candles had been employed in former filtrations and were burned free of organic matter before being used in these experiments. New Berkefeld V candles were used in each instance. In conducting the filtrations no accurate data were recorded of the temperature, duration, or pressure exercised in the process of filtration. No dilutions were made of the material used for filtration. Sterility of all filtrates was determined by plating with ordinary agar-agar mediums and subsequent incubation for 48 hours at 37.5 C.

The whole serum used represents the defibrinated, phenolized blood of hyperimmunized hogs, as obtained in the routine method of antihog-cholera-serum production employed at these laboratories. The virus is the defibrinated, phenolized blood of cholera-sick hogs, and is the strain of virus regularly used at this institute in serum testing. All products used in these experiments were retained in storage at a temperature of 48-52 F.

For testing the potency of the various substances, individual litters of pigs were employed. The animals weighed 30-60 pounds and were fair representatives of pigs generally used for testing antihog-cholera serum. The pigs received 2 c.c. each of virus. All animal inoculations were made intramuscularly.

An experiment was made with blood obtained from 2 hyperimmunized hogs.

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[1] Jour. Agric. Research, 6, p. 333.

A portion of the defibrinated blood was carbolized by adding 0.5% of carbolic acid crystals from a 5% dilution of the acid and placed in storage. Another portion was prepared according to the method of Dorset and Henley:

> To each 100 c.c. of the cool defibrinated blood add 1 c.c. of the sterile bean extract and stir to secure a uniform mixture. Allow the mixture to stand until agglutination is clearly evident. This can be determined by examining a small amount in a glass or tube. Agglutination is usually apparent within five minutes after adding the bean extract. There should then be added 1 gm. of finely powdered sodium chlorid. The salt is stirred in until dissolved, and the mixture of defibrinated blood, bean extract and salt is allowed to stand for about 15 minutes.

The agglutinated blood was centrifuged and the supernatant serum poured off and passed through a Chamberland F. filter. The whole serum and Chamberland filtrate were tested for comparative potency on a litter of 7 pigs. The test was started Oct. 5, 1916.

TABLE 1

Results of Test 1, Using Chamberland Filtrate; Amount of Virus, 2 C.c.

Date, October	Number and Weight of Pig						
	34 41 lbs.	35 35 lbs.	36 42 lbs.	37 40 lbs.	38 50 lbs.	39 49 lbs.	40 51 lbs.
	Virus Control	Whole Serum			Chamberland Filtrate		
		5 C.c.	10 C.c.	15 C.c.	5 C.c.	10 C.c.	15 C.c.
5	101.2	101.6	102.0	101.0	101.4	101.0	101.2
6	100.0	102.0	102.8	102.0	100.2	101.4	101.2
7	100.2	100.4	101.0	101.2	99.4	99.0	99.6
8	102.4	101.2	102.6	101.8	100.8	101.6	101.0
9	104.0	102.0	103.0	102.4	103.0	102.4	102.8
10	104.8	101.8	102.2	101.4	102.0	100.6	102.8
11	105.0	102.0	102.4	101.6	104.0	101.8	103.4
12	105.8	103.0	104.8	103.2	104.4	102.8	103.8*
13	106.0*	102.8	104.6	102.0	105.8	105.4	105.8
14	106.4d	101.8	102.4	101.6	106.2*	105.8	106.6*
15	106.8*	101.0	101.6	101.4	106.4*	104.8*	106.0*
16	106.4*	101.0	101.2	101.4	107.0*d	106.6*d	107.2*d
17		100.4	100.2	101.0	105.8*d	106.2*d	106.0*d
18		100.2	101.0	101.4		105.8*d	
19		101.4	101.0	101.2			
20		101.4	101.4	102.4			
21		101.2	101.0	101.2			
22		101.0	101.2	101.4			
23		100.2	101.0	100.4			
24		100.0	100.2	101.4			
25		100.2	101.0	98.6			
	Died; acute hog cholera	Remained well	Remained well	Remained well	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera

* Loss of appetite; d, diarrhea.

Test 1.—The record of Test 1 shows that the virus control pig died 11 days after inoculation and showed lesions of acute hog cholera. The pigs receiving 5, 10, and 15 c.c. of whole serum respectively, remained well throughout the test. The pigs receiving 5, 10, and 15 c.c. of Chamberland filtrate respectively, died on the 12th and 13th days after injection. Each of these pigs presented lesions of hog cholera.

From this test it is evident that the whole serum protected, while the Chamberland filtrate from the same source would not protect in the amounts employed. The centrifuged serum was not tested in this instance as it was assumed from experiments by Dorset and Henley that clear, centrifuged serum is equally as potent as the whole serum. A duplicate test was made, with material from the same source, on a litter of 10 pigs.

TEST 2.—The virus control pig died 8 days after inoculation and showed lesions of acute hog cholera. The pigs received 5, 10, 15, and 20 c.c. of Chamberland filtrate, respectively, died within 12 days following inoculations and showed symptoms and postmortem lesions of hog cholera. The pig receiving 5 c.c. of whole serum, died 13 days after injection and showed lesions of hog cholera. The pigs receiving 10, 15, and 20 c.c. of whole serum, respectively, remained healthy. The serum control, receiving 20 c.c. of a tested serum, remained well during the test.

TABLE 2

RESULTS OF TEST 2, USING CHAMBERLAND FILTRATE; AMOUNT OF VIRUS, 2 C.C.

Date, October, November	Number and Weight of Pig									
	41 35 lbs.	42 45 lbs.	43 49 lbs.	44 45 lbs.	45 47 lbs.	46 56 lbs.	47 52 lbs.	48 55 lbs.	49 55 lbs.	50 59 lbs.
	Virus Control	Chamberland Filtrate				Whole Serum				Serum Control 20 C.c.
		5 C.c.	10 C.c.	15 C.c.	20 C.c.	5 C.c.	10 C.c.	15 C.c.	20 C.c.	
12	102.4	102.0	102.8	103.2	101.8	101.4	102.2	102.2	102.8	101.2
13	101.2	101.8	102.0	102.2	102.4	101.2	102.6	102.2	102.4	102.0
14	102.4	-101.4	102.0	102.0	102.6	102.0	101.4	102.0	101.6	102.2
15	104.2	104.4	105.0	102.4	104.2	102.0	101.4	101.8	104.2	101.6
16	105.2	105.2	106.0	103.8	104.4	102.8	104.6	102.4	104.0	103.8
17	106.2	105.4	106.0	105.2	105.0	104.4	104.4	103.6	104.6	104.0
18	106.0d	104.8	105.8d	105.8	104.0	105.4	103.8	103.2	104.0	103.6
19	104.6*d	106.2*d	106.4*d	106.4*d	105.8*d	105.8	105.4	104.2	102.0	104.6
20	101.8*d	105.8*d	105.4*d	106.4*d	106.6*d	105.4*d	103.6	102.4	102.4	102.2
21		105.0*d	100.2*d	106.0*d	104.2*d	104.6d	102.0	101.8	101.6	101.4
22		106.0*d		107.4*d	104.2*d	105.2*d	101.8	102.0	101.2	101.6
23		105.4*d		106.4*d	105.8*d	104.8*d	101.6	100.4	100.6	102.2
24		106.6*d			107.2*d	107.2*d	101.8	100.2	101.0	101.2
25						106.4*d	102.0	101.4	101.0	102.6
26							102.6	101.0	99.8	101.6
27							101.4	101.0	100.6	104.8
28							101.2	101.6	101.2	102.4
29							102.0	101.6	100.4	100.6
30							101.0	100.2	101.0	100.4
31							100.4	100.4	100.0	100.2
1							100.4	100.2	101.2	101.4
2							101.0	100.2	101.2	101.4
	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera	Remained well	Remained well	Remained well	Remained well

* Loss of appetite; d, diarrhea.

The results obtained from the 1st 2 tests with Chamberland filtrate and whole serum led us to experiments using Berkefeld candles in order to determine if these candles also would hold back immune bodies, and further if the residuum would contain a concentration of these bodies. The Berkefeld filtrate thus obtained was in turn passed through a Chamberland F. filter in order to determine whether or not there were immune bodies present in the filtrate which could be restrained by a candle of this nature.

Blood for this experiment was obtained from 11 hyperimmunized hogs. One portion was agglutinated, centrifuged, and carbolized, as in the former test; 800 c.c. of the centrifuged serum were used for Berkefeld filtration and 500 c.c. of filtrate and 200 c.c. of residuum were secured; 400 c.c. of the filtrate were used for passage through a Chamberland F. candle, and from this were obtained about 125 c.c. of Chamberland filtrate and about 250 c.c. residuum. The amounts of the various filtrates and residua obtained from these filtrations are only approximate, as no special effort was made to collect the largest amounts available.

TABLE 3

RESULTS OF TEST 3 A, USING BERKEFELD AND CHAMBERLAND RESIDUUM AND FILTRATE; AMOUNT OF VIRUS, 2 C.C.

Date, January, February	Number and Weight of Pig						
	233 51 lbs.	234 46 lbs.	235 55 lbs.	236 55 lbs.	237 41 lbs.	238 47 lbs.	239 55 lbs.
	Virus Control,	Whole Serum, 10 C.c.	Centrifuged Serum, 10 C.c.	Berkefeld Residuum, 10 C.c.	Berkefeld Filtrate, 10 C.c.	Chamberland Residuum, 10 C.c.	Chamberland Filtrate, 10 C.c.
10	101.4	102.0	101.8	101.8	101.6	101.8	101.6
11	100.2	102.4	102.0	103.8	101.4	102.0	102.6
12	101.4	101.4	101.0	101.2	101.8	102.6	102.0
13	103.0	102.8	103.0	101.4	101.2	103.0	102.4
14	105.4	101.6	102.8	102.0	104.4	104.2	104.0
15	104.6	102.2	104.6	101.8	104.6	104.0	104.0
16	104.2	102.4	104.0	101.0	105.2	104.6	104.0
17	104.0	101.6	103.0	101.6	106.0*	103.4	104.2
18	105.2	102.0	104.0	101.8	106.4*	105.4	105.8
19	106.2*	101.2	102.6	100.2		107.0*	105.4*
20	104.6	101.8	103.4	100.4		106.4*	105.8*
21	105.0	102.2	104.0	101.6		106.6*	105.0*d
22	106.0*	100.6	104.4	102.6		106.4*	105.2*d
23	106.4	101.0	103.4	101.4		107.0*	
24	106.4	102.4	103.2	101.8		106.6*	
25	104.4*	103.0	103.2	101.2		107.4*d	
26	105.2*	103.4	103.0	101.2		104.8*d	
27	105.4*	101.4	102.6	101.8		101.6*d	
28	105.0*	101.2	102.8	101.4			
29	103.0*d	101.0	102.6	101.8			
30		101.4	102.2	101.2			
31		101.8	101.4	102.6			
1		101.8	101.8	101.4			
	Died; acute hog cholera	Remained well	Remained well	Remained well	Died; acute hog cholera	Died; acute hog cholera	Died; acute hog cholera

* Loss of appetite; d, diarrhea.

In this experiment whole serum, centrifuged serum, Berkefeld residuum, Berkefeld filtrate, Chamberland residuum and Chamberland filtrate were tested for comparative potency. Two litters of pigs, 7 in each, were used to determine the potency of these various products. A dosage of 10 c.c. each was used for injecting the pigs of 1 litter, and a dosage of 20 c.c. each was employed in pigs of the other litter. This test was started Jan. 10, 1917.

TEST 3A.—In the litter of pigs receiving the smaller dosage, the virus control pig died 19 days after injection with lesions of acute hog cholera. The pigs receiving 10 c.c. of whole serum, centrifuged serum, and Berkefeld residuum, respectively, remained well during the test. The pigs receiving 10 c.c. of the Berkefeld filtrate, Chamberland residuum, and Chamberland filtrate, respectively, died 8-17 days after injection with lesions of hog cholera on necropsy.

TABLE 4

RESULTS OF TEST 3 B, USING BERKEFELD AND CHAMBERLAND RESIDUUM AND FILTRATE; AMOUNT OF VIRUS, 2 C.C.

Date, January, February	Number and Weight of Pig						
	240 50 lbs.	241 60 lbs.	242 52 lbs.	243 38 lbs.	244 58 lbs.	245 54 lbs.	246 48 lbs.
	Whole Serum, 20 C.c.	Centrifuged Serum, 20 C.c.	Berkefeld Residuum, 20 C.c.	Berkefeld Filtrate, 20 C.c.	Chamberland Residuum, 20 C.c.	Chamberland Filtrate, 20 C.c.	Serum Control, 20 C.c.
10	102.0	101.2	100.6	101.8	101.0	101.2	101.4
11	101.8	103.0	104.0	102.0	102.6	101.0	102.0
12	101.6	102.0	102.2	101.2	103.2	101.0	101.0
13	102.6	102.6	101.8	102.0	103.0	101.8	102.0
14	103.0	103.2	102.4	103.0	103.8	104.0	102.2
15	103.2	103.0	101.2	102.0	104.2	102.8	101.0
16	102.6	101.8	102.0	102.4	104.0	104.2	100.4
17	102.4	101.2	102.4	102.6	104.4	103.0	101.2
18	102.4	101.8	103.0	102.0	104.8	104.2	102.0
19	101.6	100.4	102.0	102.0	104.4	102.8	101.0
20	102.0	100.2	102.0	103.4	105.8*	103.8	100.0
21	102.4	101.0	102.0	104.8	104.8	103.2	101.2
22	101.2	100.2	101.2	105.4	105.0	106.2	100.2
23	101.4	101.6	102.0	105.6*	105.8*	106.4*	100.2
24	101.8	101.4	102.0	106.0*	105.4*	107.8*	101.2
25	102.6	101.8	103.0	105.6*	105.0*		101.2
26	101.6	101.6	102.0	103.0*	104.8*		100.2
27	101.4	101.4	101.6	102.0	105.8*		101.0
28	101.2	100.2	101.6	101.8	103.2*d		100.0
29	102.0	101.6	101.2	102.0			100.2
30	102.2	101.6	101.8	101.6			101.6
31	101.4	101.0	101.2	103.8			101.2
1	102.0	101.4	101.8	103.8			101.0
	Remained well	Remained well	Remained well	Recovered, very sick	Died; acute hog cholera	Died; acute hog cholera	Remained well

* Loss of appetite; d, diarrhea.

TEST 3B.—In the 2nd litter, the pigs that received 20 c.c. of whole serum, centrifuged serum, and Berkefeld residuum, respectively, remained healthy. One pig, receiving 20 c.c. of Berkefeld filtrate, showed a marked fever and physical reaction, but eventually recovered. The pigs receiving 20 c.c. of Chamberland residuum and Chamberland filtrate, respectively, died on the 14th and 18th day following injection with lesions of hog cholera. Serum control pig which received 20 c.c. of a tested serum, remained healthy throughout the test.

The results of the experiments would indicate that the Berkefeld as well as the Chamberland filters restrain the immune bodies of antihog-cholera serum.

From Test 3 it is evident that the whole serum, centrifuged serum, and Berkefeld residuum, protected in 10 c.c. doses; while 10 c.c. of Berkefeld filtrate, Chamberland residuum, and Chamberland filtrate failed to protect the test animals against hog-cholera virus.

TABLE 5

RESULTS OF TEST 4, USING BERKEFELD RESIDUUM; AMOUNT OF VIRUS, 2 C.C.

Date, February	Number and Weight of Pig									
	285 32 lbs.	286 32 lbs.	287 33 lbs.	288 30 lbs.	289 33 lbs.	290 32 lbs.	291 32 lbs.	292 30 lbs.	293 31 lbs.	294 36 lbs.
	Virus Control	Whole Serum				Berkefeld Residuum				Serum Control 15 C.c.
		2.5 C.c.	5 C.c.	10 C.c.	15 C.c.	2.5 C.c.	5 C.c.	10 C.c.	15 C.c.	
8	103.6	103.4	103.0	103.0	103.6	103.4	103.0	103.6	103.0	103.2
9	103.0	103.4	103.2	102.6	103.4	103.6	104.4	103.8	104.2	104.0
10	102.0	101.8	102.4	101.8	102.6	101.6	102.8	102.4	101.8	102.0
11	101.8	101.4	101.6	101.6	102.0	100.2	102.0	100.4	101.0	101.2
12	104.4	104.0	103.8	102.6	102.8	104.0	102.6	102.4	102.4	102.6
13	102.0	101.2	101.8	101.6	101.0	102.8	102.6	102.6	102.8	103.0
14	103.6	104.2	101.6	102.6	103.8	102.6	102.4	102.2	102.4	102.4
15	105.0	103.4	102.4	101.8	103.2	101.6	102.2	101.8	101.8	103.4
16	105.2	105.0	103.0	104.0	104.0	101.4	101.8	101.2	102.0	102.4
17	105.6	104.4	105.4	103.6	105.0	102.0	101.8	103.2	102.8	102.0
18	106.4*	107.0*	105.6*	105.4*	104.6	101.8	102.0	102.6	102.0	101.6
19	105.8	106.8*	106.0*	103.8	103.8	103.2	101.0	104.4	102.2	101.0
20	106.2*	105.4*d	104.8*	105.0	102.0	103.0	101.2	102.8	101.6	101.8
21	106.6		103.2	103.8	102.6	104.2	101.2	102.8	101.0	101.4
22	107.0d		104.4	105.2	102.8	102.6	101.0	101.8	100.2	102.4
23	106.4*d		103.6*	104.6	102.0	101.8	101.6	101.0	101.2	101.0
24	105.4*		103.8*	104.4	102.6	102.0	101.6	101.2	100.4	100.2
25			104.2	104.0	102.8	101.6	101.0	101.8	101.0	101.2
26			104.0*	104.0	105.6	102.4	101.0	102.0	102.2	101.8
27			103.0	102.8	103.8	102.0	100.6	102.0	101.6	101.0
28			102.2	102.0	102.6	101.2	101.2	101.0	101.0	101.1
	Died; acute hog cholera	Died; acute hog cholera	Recovered, very sick	Recovered, sick	Recovered	Remained well	Remained well	Remained well	Remained well	Remained well

* Loss of appetite; d, diarrhea.

Since the Berkefeld filtrate, as demonstrated by this test, is deficient in immune bodies, it is reasonable to believe that a concentration of these bodies exists in the Berkefeld residuum. A 4th experiment was made in order to determine the relative potency of whole serum and Berkefeld residuum. A litter of 10 pigs was used for this test, which was started Feb. 8, 1917.

TEST 4.—The virus control died 16 days after the inoculation and showed lesions of acute hog cholera. Of the pigs which received 2.5, 5, 10, and 15 c.c. of whole serum, respectively, the one which received 2.5 c.c. died 12 days after injection and showed lesions of acute hog cholera; the others recovered. The pigs which received 2.5, 5, 10, and 15 c.c. of Berkefeld residuum, respectively, remained normal. The serum control, received 15 c.c. of a tested serum and remained well. In this test the Berkefeld residuum protected more satisfactorily, in these amounts, than did the whole serum.

SUMMARY

The results obtained in the first 2 tests indicate that the immune bodies of antihog-cholera serum, when prepared as stated, are restrained by filtration through Chamberland F. filters.

The 3rd test shows that the Berkefeld as well as the Chamberland filter restrains immune bodies of antihog-cholera serum.

When Berkefeld filtrate was in turn passed through a Chamberland F. filter, the resulting residuum was not sufficiently potent in a dose of 20 c.c. to protect the test animal against simultaneous inoculation with 2 c.c. of hog-cholera virus.

In the 4th test the Berkefeld residuum protected more satisfactorily than did the whole serum.

We were unable to obtain a potent, bacteria-free antihog-cholera serum by filtration through either Berkefeld or Chamberland F. candles.

Blood serum being rich in proteins, adsorption of these according to Bechhold, Rosenthal, and others,[2] always takes place in the filter pores, so that finally the filtration is actually through a colloid filter. With this condition existing, the 1st portion of the filtrates passing through the candles may have contained the immune bodies of antihog-cholera serum, while the latter portion may have been free of these bodies.

While it may be possible to produce a potent, bacteria-free antihog-cholera serum by filtration of serum obtained as the result of normal clotting or by centrifuging defibrinated blood, experiments to determine this were not conducted.

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[2] Am. Vet. Review, 1914, 16, p. 134.